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(54)   **Stent for dilating a stenotic lesion of a blood vessel**

(57)   A stent having long-term antithrombogenicity and radiopacity whose location and geometry can be radiographically confirmed is provided. The production method is also provided. The stent of the invention has an antithrombogenic agent covalently bonded on its surface, and the antithrombogenic agent is immobilized onto the stent surface through a coupling agent having at least two amino groups and a cross-linking agent having at least two aldehyde and/or epoxy groups. The stent of the invention may also have radiopaque metal plating on at least a part thereof.

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Description

BACKGROUND OF THE INVENTION

5   This invention relates to a stent used for dilating a stenotic lesion of a blood vessel.

PTCA (percutaneous transluminal coronary angioplasty) is a widely adopted treatment in the case of coronary artery stenotic diseases such as stenocardia and myocardial infarction. In PTCA, the stenotic lesion of the blood vessel (coronary artery) is pushed open from its inside to enable the blood to flow therethrough.

In order to avoid restenosis of the blood vessel after the blood vessel dilatation, an expandable intraluminal graft

10   ("stent") as disclosed in US Patent 4,733,665 has been developed.

A stent is a cylindrical medical device used to maintain the inside opening of a body duct such as a blood vessel and bile duct. The stent is introduced into the body duct in compacted or folded thin state. When the stent is positioned in the target site (stenosis lesion), the stent is expanded and pressed into place against the inner wall of the lumen and stands there to serve as a structural support for the lumen.

15   There are two mechanisms for the stent expansion. One is the mechanism wherein the stent is pushed radially outwardly from its inside by an external force, namely, by placing a balloon inside the stent and inflating the balloon. The other is the mechanism wherein a stent capable of restoring its original shape is folded in a compact shape, and the force retaining such compact shape is removed to allow for the stent to self-expand in a radially outward direction by its own restorative force.

20   The stent of the first type which is expanded in a radially outward direction by the external force of the inflated balloon is called a balloon-expanded stent, and the stent of this type is not self-expandable. The balloon-expanded stent is made of a metal material which is less likely to restore its original shape once the stent is expanded, and in view of biological safety, the preferred materials are inactive in a body such as stainless steel, tantalum, and the like.

The stent that expands by its own restorative force is called a self-expandable stent, and the stent of this type is

25   provided with a constriction means and is self-expandable. For example, the stent may be constricted and accommodated in a tube having an outer diameter smaller than the inner diameter of the target lesion, and once the distal end of the tube has reached the target lesion, the stent may be pushed out of the tube to allow its self-expansion. As described above, the self-expandable stent should be capable of restoring its original shape, and therefore, such stent is made from stainless steel, a superelastic alloy, or a shape memory alloy (nickel-titanium alloy, in the latter two cases).

30   Of the self-expandable stents, the one employing a super-elastic alloy (shape memory alloy) is disclosed in JP-B-5-43392.

As described above, most of currently available stents are made of a metal. Metal has been selected in view of its inherent physical properties and particular function as well as safety and compatibility with the tissue. Metal, however, is an utterly foreign matter from the standpoint of blood, and the blood induces thrombus formation and platelet acti-

35   vation to induce vascular smooth muscle cell (VSMC) migration and proliferation.

Since the stent generally has a complex net structure, the placement is always involved with some risk of narrowing or blockage of the blood vessel by thrombus formation or vascular smooth muscle cell migration and proliferation, and this is the reason why the patient is administered with an antithrombotic agent such as heparin or warfarin for approximately two weeks following the stent placement until the inner surface of the stent is substantially covered with he-

40   mangioendothelial cells. Such administration of the antithrombotic agent may result in the occurrence of a bleeding complication from the artery at the puncture site or from the peripherals.

If restenosis shortly after the stent placement induced by the vascular smooth muscle cell migration and proliferation through the platelet activation could be avoided, and dosage and period of the antithrombotic administration could be reduced or nulled, the results should be shorter lengths of hospital stay and decreased bleeding complications.

45   In the meanwhile, various methods have been developed for the purpose of rendering medical devices antithrombogenic.

US Patent 3,810,781 discloses a method for imparting antithrombogenicity to a surface of plastic resin wherein a cationic surfactant is adsorbed on the plastic surface, and heparin which has been cross-linked with glutaraldehyde is ionically bonded to the surfactant.

50   US Patent 4,118,485 discloses a method for imparting antithrombogenicity to a surface of substrate of a plastic resin, glass, or metal wherein the substrate surface is coated with a heparin-quaternary amine complex compound, and the surface is further treated with glutaraldehyde to form a Schiff base to thereby impart antithrombogenicity with the substrate surface. There is also disclosed a method wherein the substrate surface is coated with a heparin-quaternary amine (benzalkonium chloride) complex compound to impart antithrombogenicity to the substrate surface.

55   In these methods, however, the heparin, the heparin-quaternary amine compound, or the heparin-quaternary amine-glutaraldehyde compound is attached to the substrate surface through IPN (interpenetrating network) structure or intermolecular force, and therefore, the heparin-substrate bond is not sufficiently strong as in the case of covalent bond.

EP-A-679373 discloses a method wherein the stent is imparted with antithrombogenicity by vacuum depositing a Parylene resin coating on the stent.

JP-B-6-38851 discloses various methods for covalently bonding heparin or a derivative thereof to the surface of various plastic resin materials. In these methods, the heparin derivative is covalently bonded to the substrate surface, and the heparin derivative is unlikely to be removed from the surface. However, in the case of a metal material which is less active than the plastic material, it is unknown whether such heparin derivative can be covalently bonded to the metal surface by the same procedure. Even if such heparin derivative could be covalently bonded to the metal surface of a stent, it is still unknown whether the heparin derivative can effectively suppress the vascular smooth muscle cell migration and proliferation.

Stainless steel, the most widely adopted material for a stent, is radiotranslucent, and since a stent has a net structure, and the width of the stainless steel item which constitutes a net has a thickness of 0.2 mm, it has been quite difficult to confirm the exact location and geometry of the radiotranslucent stainless steel stent during and after surgery. A radiopaque marker has been provided on the catheter to indicate the location of the stent. Such radiopaque markers on the catheter, however, have been far from being satisfactory since there is a considerable risk of the stent becoming displaced from the predetermined site on the catheter. In addition, incapability of direct confirmation of the catheter geometry often resulted in failure to discover insufficient stent expansion and "elastic recoiling" which is the recovery of the once expanded stent to its initial unexpanded state by the restoration tendency to some extent inherent in all metal materials.

In order to obviate such a situation, EP-A-679372 discloses a stent provided with a radiopaque marker. A stent plated with a metal for the purpose of imparting radiopacity, however, suffers from the risk of inducing the narrowing and blocking of the blood vessel at the site where it is placed.

SUMMARY OF THE INVENTION

In view of the above-described situation, an object of the present invention is to provide a stent having a long-term antithrombogenicity as well as excellent tissue reformability.

Another object of the present invention is to provide a stent whose location and geometry can be confirmed by X-ray irradiation.

Another object of the present invention is to provide a method for producing such a stent.

Such objects of the present invention are attained by the present invention summarized in the following items (1) to (8).

(1) A stent for dilating stenotic lesions of blood vessels which comprises a substrate, wherein an antithrombogenic agent is covalently bonded to the surface of said substrate through a coupling agent having at least two amino groups and a cross-linking agent having at least two aldehyde and/or epoxy groups, said surface being treated with an oxidizing agent.

(2) A stent according to the above item (1) wherein said oxidizing agent is ozone.

(3) A stent according to item (1) or (2) wherein said antithrombogenic agent is aminated heparin.

(4) A stent according to one of items (1) to (3) wherein said substrate comprises stainless steel.

(5) A stent according to one of items (1) to (4) wherein said coupling agent is at least one member selected from the group consisting of polyethylene imine, polyethylene glycol diamine, ethylenediamine, and tetramethylenediamine.

(6) A stent according to one of items (1) to (5) wherein said cross-linking agent is glutaraldehyde or ethylene glycol glycidylether.

(7) A stent according to one of items (1) to (6) wherein said (metal) substrate is plated with a radiopaque metal on at least a part thereof.

(8) A stent wherein the surface that is brought in contact with the blood is rendered antithrombogenic, and the surface that is not brought in contact with the blood is rendered radiopaque.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. (a) and FIG. 1 (b) are fold-out views of an embodiment of the stent according to the present invention.
FIG. 2 is a perspective view of an embodiment of the stent according to the present invention
FIG. 3 is a cross-sectional view showing one unit of the stent of FIG. 2.

DETAILED DESCRIPTION OF THE INVENTION

The stent of the present invention comprises a substrate of any desired material such as a resin or a metal.

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Preferably, the material used for the stent substrate is a biocompatible material such as stainless steel, tantalum, superelastic nickel-titanium (Ni-Ti) alloy, or a thermoplastic polymer.

The stent of the present invention is not limited with regard to its shape. However, it is preferably expandable in a radial direction from the constricted state to the expanded state, and flexible in an axial direction.

The stent may typically have a cylindrical shape in the form of a coil, net, or bellows, and the stent may comprise either a single unit or a train of two or more units. FIG. 1 is an embodiment of the stent in its constricted state. (a) is a fold-out view of the constricted state, and (b) is a fold-out view of the expanded state.

A perspective view of the exemplary stent of the present invention is shown in FIG. 2. Stent 1 of the present invention has an antithrombogenic agent 2 covalently bonded thereto, and a radiopaque metal plated layer 3 deposited on at least a part of the stent. In the embodiment shown in FIG. 2 whose part is cross-sectionally shown in FIG. 3, the antithrombogenic agent 2 is covalently bonded to the surface of the stent that comes into contact with the blood, and the radiopaque metal plated layer 3 is deposited on the stent surface that will not come into contact with the blood. In other words, the outer surface of the stent is deposited with a metal plated layer 3, and the backside and flank of the outer surface of the stent are covalently bonded with the antithrombogenic agent 2.

The surface of the stent is treated to impart either antithrombogenicity or X-ray opacity. The stent surface may be treated by either or both of such surface treatments. When treated by both surface treatments, the surface is preferably rendered radiopaque first, and then, antithrombogenic.

Exemplary radiopacity treatments include plating of a radiopaque metal such as gold, argent, platinum, iridium, tantalum and the like on the stent surface. It has been thus far considered difficult to effect any further surface treatment on the radiopaque metal-plated surface since many radiopaque metals are noble metals and the radiopaque metal-plated surface is inactive, and no attempt has been made to render the metal-plated surface thromboresistant. It is the present invention that for the first time enables the provision of a stent having both the radiopacity and antithrombogenicity by a convenient process, namely, by metal plating the stent on a desired surface, and subsequently effecting the antithrombogenic treatment on another desired surface. The stent is preferably metal-plated on its outer surface or a part thereof that will not come into contact with the blood. In view of X-ray opacity, the stent is preferably plated along its full length to enable satisfactory visibility. The preferable radiopaque metal is gold in view of its excellent stability. Each metal has inherent radiopacity, and therefore, the metal should be plated to a thickness that would enable angiography after the stent placement. In the case of gold, the radiopaque layer is preferably plated to a thickness of from 10 to 40 μm.

When a stent of cylindrical shape is plated on its outer surface, the plating should be conducted after masking the inner surface to avoid the metal deposition thereto. Preferably, a metal cylinder pipe is bunged at both ends so that the plating liquid cannot enter therein. Subsequently, the sealed cylinder pipe is metal-plated, and then the cylinder pipe is given a shape of a stent by stamping or laser treatment.

Irrespective of whether or not the stent is rendered radiopaque as describe above, the stent is rendered antithrombogenic as described below.

(1) The surface of the stent is first treated with an oxidizing agent.

Exemplary oxidizing agents are halogen gas, ozone, chromic acid derivatives and the like, ozone being preferred. Treatment with ozone is one of the strongest oxidization treatments, and therefore adequate for activating chemically inactive surface of a metal such as stainless steel.

In the course of oxidation by ozone, the substrate surface is not only chemically activated but also physically activated to result in surface roughening. The minute surface irregularities formed will serve as an anchor for the coupling agent used in the heparin immobilization. The impurities on the surface which may interfere with the anchoring of the coupling agent are also decomposed through the oxidation by ozone treatment, enabling strong heparin immobilization.

Ozone is produced in an ozone generator by oxidation of oxygen, and the ozone treatment is effected by bringing the thus generated ozone in contact with the stent. The ozone treatment is carried out at an ozone concentration, oxygen (ozone) flow rate, reaction temperature and reaction period adequately selected according to the material of the stent treated. When the surface treated is a metal surface, the ozone treatment may be carried out under severer conditions than in the case of treating an organic resin surface.

The ozone concentration is preferably in the range of from 20 to 100 mg/l. An ozone concentration of less than 20 mg/l will result in an insufficient surface activation while an ozone concentration of more than 100 mg/l may involve some risk of non-uniform reaction.

The oxygen (ozone) flow rate is preferably in the range of from 50 to 1,000 ml/min. A flow rate of less than 50 ml/min may result in localized reaction, and a flow rate of more than 1,000 ml/min is uneconomical since some of the ozone will not be involved in the surface activation.

The reaction temperature may be determined by raising the temperature within the range of from 0 to 70°C according to the nature of the material if the stent surface is non-oxidizable at normal temperature.

The reaction period is preferably within the range of from 30 to 120 minutes. The reaction will be difficult to control if the ozone treatment is carried out within 30 minutes. A reaction period of longer than 120 minutes is too long and inefficient.

(2) Next, an antithrombogenic agent is covalently bonded to the stent surface through a coupling agent having two or more amino groups and a cross-linking agent having two or more aldehyde or epoxy groups.

The activated surface is reacted with a coupling agent having two or more amino groups which temporarily enable ionic bonding of the antithrombogenic molecule (preferably, the heparin molecule) to the activated surface. Exemplary coupling agents include polyethylene imine, polyethylene glycol diamine, ethylenediamine, and tetramethylenediamine.

The heparin molecule is brought into contact with the substrate surface having the thus attached amino groups to ionically bond the heparin to the substrate. The heparin is temporarily attached to the substrate surface for covalent bonding in the subsequent step.

The heparin molecule that is brought into contact with the substrate surface may be the heparin molecule itself. The heparin molecule, however, is preferably the one having a part of its N-sulfate group moieties desulfated and converted to primary amine moieties. The percentage of the primary amino group in the heparin molecule, however, may be preferably adjusted to the range of from 5 to 25% since heparin is inactivated in proportion to the degree of such desulfation. When heparin or aminated heparin is used for the antithrombogenic agent, the stent will retain sufficient antithrombogenicity for about two weeks after the stent placement in the lesion. Use of the heparin or the aminated heparin also results in high restoration of the body tissue.

The antithrombogenic agent may alternatively comprise a compound which is antithrombogenic and capable of suppressing smooth muscle cell migration such as a calcium antagonist, an inhibitor of the angiotension-converting enzyme, or an antagonist of the receptor of platelet membrane glycoprotein (IIb-IIIa antagonist). The antithrombogenic agent should also be capable of ionically binding to the amino group.

a) The coupling agent may be attached to the substrate surface by applying the solution of the coupling agent to the substrate surface through coating or dipping. The solution may be adjusted to a pH of from 4 to 12 since a pH outside this range may result in the outflow of the ion in some substrate metal material to result in the loss of activated surface. The reaction temperature is preferably in the range of from 0°C to less than 80°C. A reaction temperature of lower than 0°C results in reduced reactivity, while a reaction temperature above 80°C is associated with the risk of denaturation of the coupling agent. The reaction period is preferably from 10 minutes to 24 hours. A reaction period of less than 10 minutes results in insufficient reaction, while a reaction period of more than 24 hours is associated with the risk of denaturation of the coupling agent at some pH or temperature.

Next, the antithrombogenic agent, preferably heparin, is ionically bonded to the substrate by means of applying the heparin solution to the substrate surface having the coupling agent attached thereto through coating or dipping. The solution applied is preferably adjusted to a pH of from 2 to 5 since a pH of less than 2 results in a reduced heparin stability while a pH in excess of 5 results in the reduction of the positive charge on the substrate surface, leading to a reduced amount of the heparin bonded. The reaction temperature is preferably in the range of from 0 to 80°C since a reaction temperature of lower than 0°C results in a significantly reduced ionic bonding rate of the heparin while a reaction temperature of higher than 80°C results in a reduced heparin stability. The reaction period is preferably from 10 minutes to 24 hours since a reaction period of less than 10 minutes results in an insufficient ionic bonding while a reaction period of more than 24 hours results in a reduced heparin activity. The heparin concentration is preferably 0.05% or more and preferably equal to or lower than the saturation concentration.

b) Next, a compound having at least two aldehyde or epoxy groups is reacted as a cross-linking agent between the coupling agent and the heparin molecule in order to covalently bond the antithrombogenic agent, preferably heparin, to the substrate surface. Exemplary crosslinking agents are glutaraldehyde and ethylene glycol diglycidylether. When a dialdehyde derivative is used as the cross-linking agent, the covalent bond formed is unstable, and a subsequent stabilization by reduction is required.

The formation of a covalent bond by a cross-linking agent may be effected by applying a solution of the cross-linking agent to the substrate surface through coating or dipping. The solution of the cross-linking agent preferably has a concentration in the range of from 0.1 to 0.5%, since a concentration of less than 0.1% results in insufficient cross-linking and a concentration higher than 0.5% adversely affects the heparin activity. The pH is preferably in the range of from 2 to 5 since a pH of less than 2 results in a reduced heparin stability, and a pH higher than 5 results in the rapid release of the ionically bonded heparin from the substrate before cross-linking. The reaction temperature is preferably in the range of from 0 to 60°C since a reaction temperature lower than 0°C results in an insufficient reaction and a reaction temperature higher than 60°C will induce heparin inactivation. The reaction period is preferably in the range of from 30 minutes to 24 hours since a reaction period shorter than 30 minutes

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is insufficient for completion of cross-linking and a reaction period longer than 24 hours will induce heparin inactivation.

When a dialdehyde derivative is used as the cross-linking agent, the covalent bond formed is unstable as mentioned above. The unstable bond formed (Schiff base) is subsequently stabilized by applying a solution of a reducing agent through coating or dipping. In a preferred embodiment, the reducing agent is added to the solution which is undergoing the crosslinking reaction, and then, the heparin immobilization can be completed as a reaction within the reaction cascade and the reduction can be completed with no breakage of the unstable covalent bond. The reducing agent in an amount at least stoichiometric with the bond to be reduced is used for the reduction. Addition of the reducing agent in a smaller amount at a higher concentration is desirable for avoiding the change in reaction conditions such as the temperature and the concentration of the cross-linking agent. The reaction period should be limited to no more than 2 hours to thereby avoid heparin inactivation.

The present invention is described in further detail by referring to Examples and Comparative Examples, which by no means limit the scope of the invention.

EXAMPLES

Example 1

A stainless steel plate (material: SUS 316L) of 1 mm thick was cut into samples of 8 mm x 8 mm. The sample plates were treated with ozone at an ozone concentration of 88 mg/l and an oxygen flow rate of 800 ml/min for reaction periods of 0, 30, 60, 120 and 180 minutes. The sample plates were evaluated for their surface elementary oxygen percentage by electron spectroscopy for chemical analysis (hereinafter abbreviated as ESCA). The results are shown in Table 1.

Table 1

Percentage of elementary oxygen on ozone-treated samples					
Ozone reaction period, min.	0	30	60	120	180
Percentage of elementary oxygen	40.49	51.12	60.29	61.90	60.92

The percentage of elementary oxygen becomes substantially constant at a reaction period of 60 minutes, and it was likely that the oxide on the sample surface had become saturated at the reaction period of 60 minutes. Therefore, a reaction period of more than 60 minutes would be unnecessary while a reaction period of less than 60 minutes would result in an insufficient reaction since the percentage of the oxide on the stent surface is still increasing. By measuring the percentage of elementary oxygen as described above, it has been possible to confirm that the substrate surface is activated by ozone treatment and to determine the optimal conditions for the ozone treatment of the SUS 316L. Optimal conditions can be determined for other metal substrate materials in a similar manner.

Example 2

The sample plates of Example 1 were immersed in 0.5% aqueous solution of polyethylene imine (hereinafter abbreviated as PEI) for 5 hours to immobilize PEI on the sample surface. The samples were evaluated by an abrasion test by rubbing the sample surface with a polyethylene terephthalate film and analyzing each sample by ESCA to thereby confirm that the PEI is not only present in an unbound state, but bonded to the sample surface. The element measured in ESCA was nitrogen from PEI. The results are shown in Table 2.

Table 2

Percentage of elementary nitrogen on each sample surface					
Ozone reaction period, min.	0	30	60	120	180
Before the abrasion test	11.43	15.76	17.14	14.27	12.10
After the abrasion test	4.87	10.75	12.62	10.63	11.04

Removal of the PEI was clear in the non-ozone treated sample (the sample with the ozone reaction period of 0 minute), and it was confirmed that a maximum amount of PEI is immobilized when the sample plates are ozone-treated for a reaction period of 60 minutes. As described above, the substrate surface is effectively activated by ozone treatment, and the optimal conditions determined in Example 1 for SUS 316L are effective for the immobilization of PEI.

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Example 3

In order to ionically bond the heparin to the sample substrate, a solution of heparin having a part of its N-sulfate group moieties desulfated and converted to primary amine moieties (synthesis of the aminated heparin was conducted in accordance with JP-B-8-38851) was prepared by dissolving the aminated heparin in 50mM succinate buffer, pH 4.0 to a concentration of 0.05%, and the sample substrates of Example 2 which have been ozone-treated for 60 minutes were immersed in the solution at 45°C for 2 hours. The samples of this step (in which heparin is attached to the substrate through an ion bond) are designated samples A.

All of the samples A (except for the samples which are not subjected to further treatment) were subjected to a series of treatments as described below to attach the heparin to the substrate through a covalent bond.

The samples A were immersed in 2 ml of 0.1%, 0.2%, 0.3% and 0.5% aqueous solutions of glutaraldehyde (which serves as the cross-linking agent) at 55°C for 2 hours, respectively. After the immersion, 0.2 ml of aqueous solution of sodium cyanoborohydride at a concentration 20 times higher than the glutaraldehyde solution was added to each glutaraldehyde solution, and the solution was thoroughly stirred and allowed to stand at 55°C for 2 hours to reduce the bonding sites. The samples were then immersed in distilled water at 55°C for 1 hour to remove the excessive cross-linking agent and the reducing agent. The resulting samples were designated samples B1, B2, B3 and B4 samples.

The above-described procedure was repeated except that the cross-linking and reducing reactions were conducted at 4°C. The samples were designated C1, C2, C3 andC4 samples.

The samples were washed by circulating physiological saline at 37°C for 3 days at a flow rate of 410 ml/min, and the samples were evaluated for their heparin activity remaining on their surface by using a commercially available measurement kit (Test-Team Heparin kit, Daiichi-Kagaku Yakuhin). The results are shown in Table 3.

Table 3(1)

Heparin activity remaining on the sample surface [IUx10^{-3}/cm^2]					
	A	B1	B2	B3	B4
Unwashed	153.7	129.6	86.1	45.9	6.7
3 days	44.2	81.6	54.2	27.7	-

Table 3(2)

Heparin activity remaining on the sample surface [IUx10^{-3}/cm^2]				
	C1	C2	C3	C4
Unwashed	151.9	161.0	160.3	101.6
3 days	105.3	131.6	132.1	-

Effects of cross-linking for the heparin immobilization were evident from the results of the non-cross-linked sample A which underwent clear reduction in the heparin activity. Optimal cross-linking in Example 3 resulted at a temperature of 4°C and a glutaraldehyde concentration of 0.2% or 0.3%.

Example 4

The sample C3 which exhibited the optimal results in Example 3 and the contrast sample (untreated stainless steel. sample, SUS 316L) were subjected to an ethylene oxide gas sterilization (hereinafter referred to as EOG sterilization), and then to a platelet expandability test. The sterilization was conducted under the conditions of the temperature of 50°C, humidity of 65%, EOG pressure of 0.7 kg/cm^2, reaction period of 3 hours, and EOG was removed by repeating 3 times the process of reducing pressure and introducing air. The samples were left for 7 days after the sterilization. The results are shown in Table 5. Reactivity of the sample surface with the platelets can be determined from the results.

The procedure of the platelet expandability test and its significance are as described below.

The sample surface was brought into contact with platelet-rich plasma (hereinafter referred to as PRP) containing a preliminarily adjusted number of platlets for a fixed time, and the samples were evaluated for their antithrombogenic level by the number and morphology of the platelets that became attached to the sample surface.

More illustratively, human vein blood (having 3.8% sodium citrate added thereto) was centrifuged to separate PRP (platelet-rich plasma), and a further centrifugation was conducted to obtain platelet-poor plasma (hereinafter referred

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to as PPP). The thus prepared PRP and PPP were used to prepare a PRP wherein the number of the platelets contained is adjusted to $10 \times 10^4/\mu l$. 200 μl of the adjusted PRP were dropped on to an 8 x 8 mm sample, and a polystyrene petri dish was placed on the droplet to adjust the liquid thickness to 2 mm. After leaving to stand for 30 minutes, the sample was washed, fixed with 1% aqueous solution of glutaraldehyde, and observed and photographed with an electron microscope. The number of platelets adhered to the unit area sample was counted on the electron microphotograph for each morphological category as described below. The results are shown in Table 4.

Table 4

Results of platelet expanding ability test [/0.03 mm^2]			
	Type I	Type II	Type III
Contrast (SUS 316L)	1	7	37
Heparin coated sample (C3)	13	7	0
Type I: The platelets underwent a change in their morphology from disk-like normal morphology to spherical morphology. Few pseudopods were observed. Type II: Several or more pseudopods were observed. The cells started to expand. Type III: the cells fully expanded and collapsed.			

Surface reactivity of the stainless steel substrate to the platelets was reduced by the heparin coating to result in the antithrombogenicity of the substrate.

Example 5 and Comparative Examples 1 and 2

Nine stainless steel pipes (material: SUS 316L; length: 20 mm; outer diameter: 1.4 mm; wall thickness: 0.1 mm) were laser-worked into balloon-expandable stents. Of these stent samples, 3 stent samples were coated with heparin under the conditions of Example 3, sample C3 (hereinafter referred to as HC (heparin-coated) samples; example 5); and 3 stent samples were coated by a conventional technique, namely, with heparin-benzalkonium (heparin-dimethylstearylbenzyl chloride) for contrast purposes (hereinafter referred to as HB (heparin-benzalkonium) samples; comparative example 1). The remaining 3 stent samples were used with no further treatment (hereinafter referred to as NH (non-heparin) samples; comparative example 2).

The stents were washed by circulating physiological saline at 37°C for 14 days at a flow rate of 410 ml/min. The stents were evaluated for their heparin activity by repeating the procedure of Example 3. The results are shown in Table 5.

Table 5

Heparin activity after washing [IU x 10^{-3}/cm^2] (n = 3)			
Sample	HC	HB	NH
Activity	49.3	0.3	0

The results demonstrate that the activity can be retained for a prolonged period by heparinizing the substrate surface in accordance with the method of the present invention.

Example 6 and Comparative Examples 3 and 4

The procedure of Example 5 and Comparative Examples 1 and 2 was repeated to prepare 3 types of stents (3 stents/type). The resulting 9 stents were sterilized by EOG sterilization under the conditions of Example 4, and the stents were allowed to stand for 7 days to remove the EOG. The stents were then inserted into the abdonminal aorta (blood vessel diameter, 3 mm) of 9 white rabbits (Kitayama-Rabeth), each weighing from 2.5 to 3.0 kg, from their femoral artery by using a balloon catheter, and the catheter was placed in the abdominal aorta after the expansion. The balloon used had an inflated diameter of 3 mm. Anticoagulation treatment by heparin administration was conducted during the operation. Two weeks after the operation, the animals were administered with heparin, and sacrificed by excessive administration of anesthetic to remove the stent. Little or no thrombus adhesion was found in the case of three stents of HC samples. On the other hand, in the HB and NH stent samples, a large amount of thrombus (about to induce stenosis) was observed in 2/3 of the stents, and thrombus adhesion of moderate degree was found on the remaining 1/3 of the stents.

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The blood vessel within which the stent had been placed was embedded in paraffin to prepare a soft tissue pathological specimen, and the specimen was examined for hemangioendothelial cells. No neoblastic smooth muscle tissue was found in the cases of the HC and HB stent samples while neoblastic signs were noticed in one case of the NH stent samples. The results are shown in Table 6.

Table 6

Results of animal experiment (n = 3)			
Sample	HC	HB	NH
Thrombus	◎	×	×
New tissue	◎	◎	Δ

◎: thrombus or neoblastic signs were noticed in 0/3 of the cases.
Δ: thrombus or neoblastic signs were noticed in 1/3 of the cases.
×: thrombus or neoblastic signs were found in all 3 cases.

Example 7

Of 2 stainless steel pipes (material: SUS 316L; length: 20 mm; outer diameter: 1.4 mm; wall thickness: 0.1 mm), one pipe was electroplated on its outer surface with gold to a thickness of 30 μm. The stainless steel pipes were laser-worked into balloon-expandable stents, and the stents were coated with heparin under the conditions of Example 3, sample C3. The stents were then expanded with a balloon to evaluate visibility by radiography. The gold-plated stent was radiographically visible not only on its outer contour but also its laser-cut design while the non-plated stent was substantially radiotranslucent.

In the foregoing Examples, the stent of the present invention has been described by featuring the case of a stainless steel stent. It should be noted, however, that the antithrombogenic treatment of the present invention may be effected on a balloon-expanded stent which has been plated with gold on its entire surface, or an expandable stent which has been coated with an appropriate synthetic resin (see JP-B-6-38851 for the type of resins that can be used for such coating) after optional radiopacity treatment.

The antithrombogenic and/or radiopaque treatment of the present invention may be carried out not only on stents, but also on various metal medical materials and equipment which are brought into contact with blood.

BENEFITS OF THE INVENTION

As described above, the stent of the present invention having an antithrombogenic agent immobilized on its surface has realized a strong bond between the heparin molecule and the substrate surface which is superior to the conventional coating of heparin-benzalkonium, and therefore, the antithrombogenic activity is maintained under the severe conditions of blood flow. As a consequence, use of the stent of the present invention will avoid restenosis due to adhesion of the thrombus of the lesion after the stent placement, and alleviate clinical treatment such as administration of the antithrombotic agent.

The immobilization of the antithrombogenic agent also reduces the event of restenosis through vascular thickening induced by vascular smooth muscle cell migration and proliferation.

In addition, the substantially radiotranslucent stent can be simultaneously rendered antithrombogenic and radiopaque in a simple manner by plating a radiopaque metal on the stent before the immobilization of the antithrombogenic agent on the stent surface.

Claims

1. A stent for dilating stenotic lesions of blood vessels which comprises a substrate, wherein an antithrombogenic agent is covalently bonded to the surface of said substrate through a coupling agent having at least two amino groups and a cross-linking agent having at least two aldehyde and/or epoxy groups, said surface being treated with an oxidizing agent.

2. A stent according to claim 1, wherein said oxidizing agent is ozone.

3. A stent according to claim 1 or 2, wherein said antithrombogenic agent is aminated heparin.

4. A stent according to one of claims 1 to 3, wherein said substrate comprises stainless steel.

5. A stent according to one of claims 1 to 4, wherein said coupling agent is at least one member selected from the group consisting of polyethylene imine, polyethylene glycol diamine, ethylenediamine, and tetramethylenediamine.

6. A stent according to one of claims 1 to 5, wherein said cross-linking agent is glutaraldehyde or ethylene glycol diglycidylether.

7. A stent according to one of claims 1 to 6, wherein said surface is plated with a radiopaque metal on at least a part thereof.
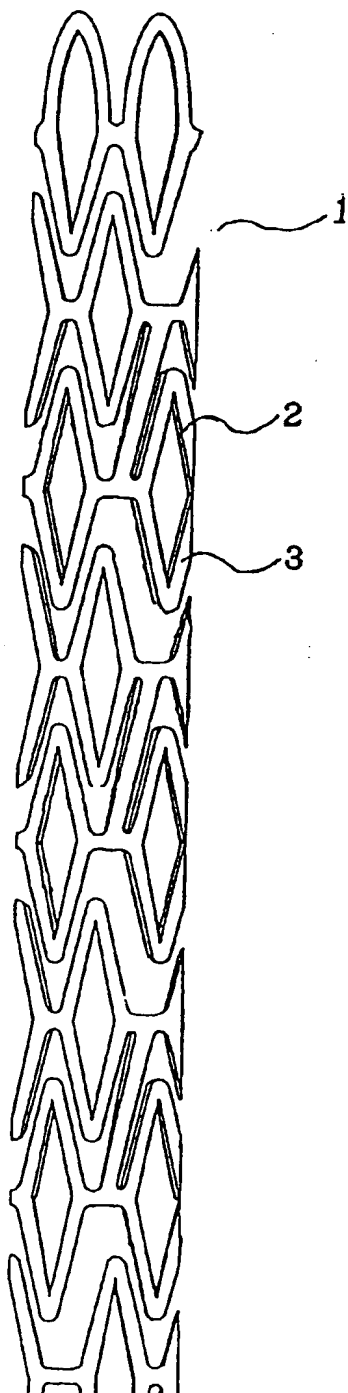
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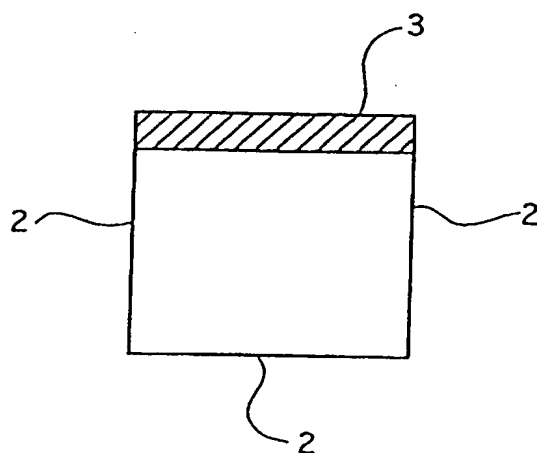
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F I G. 1

(a)   (b)
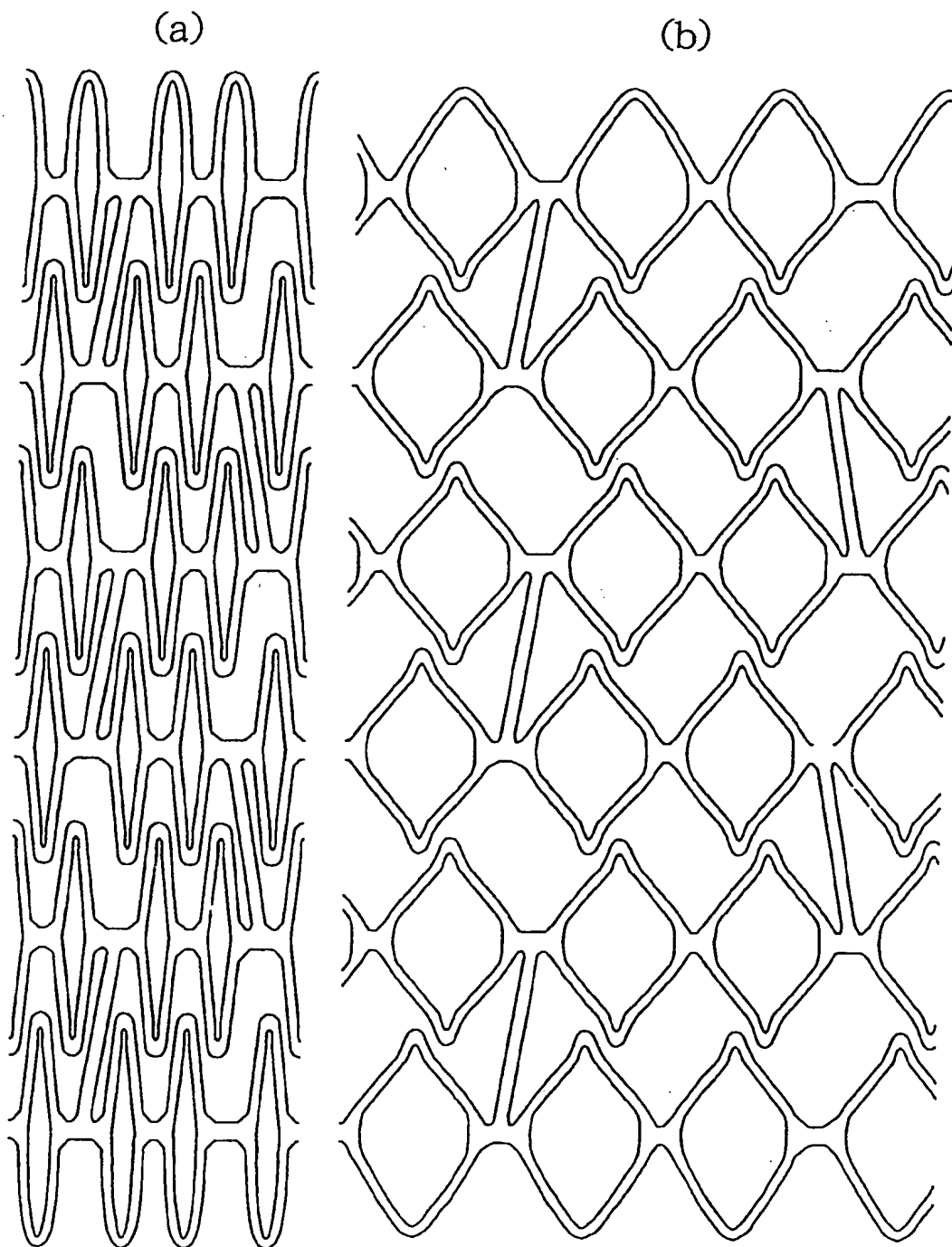
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FIG. 2

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F I G. 3

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	European Patent Office	EUROPEAN SEARCH REPORT	Application Number EP 97 40 2240

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication. where appropriate. of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	EP 0 351 314 A (TERUMO) * the whole document *	1-7	A61F2/06 A61L33/00
Y	EP 0 679 372 A (ADVANCED CARDIOVASCULAR SYSTEMS. INC) * column 2. line 26 - line 45 *	1-7	
A	US 5 356 433 A (ROWLAND ET AL) * column 4, line 22 - column 6. line 11 *	1.3.4	
A	PATENT ABSTRACTS OF JAPAN vol. 018. no. 381 (C-1226), 18 July 1994 & JP 06 105902 A (TERUMO CORP), 19 April 1994, * abstract *		

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A61F A61L

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	8 January 1998	Smith, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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